ON THE ROLE OF ACID IN THE DIGESTION OF CERTAIN RHIZOPODS.

By John C. Hemmeter, M. D.,[1]

In the "Annales de l'Institut Pasteur," for 1890 and 1891, there are two papers by M. Felix le Dantec on "Researches on the intracellular digestion among the Protozoa," which are detailed accounts of systematic experimentation concerning the occurrence of acid in the digestive vacuoles of Protozoa.

In 1889, E. Metchnikoff published a discussion of the reaction of plasmodia to ingested litmus, also in the "Annales de l'Inst. Pasteur."

Miss M. Greenwood and E. R. Saunders, in the "Journal of Physiology," Vol. XVI, 5 and 6, 1894, have published an exhaustive account of the function of acid in Protozoan digestion, of which the following brief abstract is considered necessary before proceeding to the original part of this report.

It was found that while these protozoa ingest solid matter constantly and promiscuously, such matter has a determinate fate. If it is innutritious it is ejected after lying in contact with the animal's substance for a length of time which varies with many changing conditions. Nutritious matter, on the other hand, during enclosure in food vacuoles undergoes profound change, and this change is effected by something passed out of the protoplasm into the vacuole, acting in a fluid medium and by its presence making that medium deserving of the name "secretion." In Actinospaerium, also, and in *Amoeba proteus*, digestion in like manner is effected, not by direct contact with the acting protoplasm but by some constituent of a fluid, the formation of which the presence of food alone is potent to bring about. These protozoa depend upon the solution of proteid for nourishment. Starch undergoes no digestive change, and the value of ingested fat globules is doubtful.

The following is a report on the role of acid in these digestive vacuoles. For method of observation, it may be briefly

[1] Phil. D. Etc. Baltimore, Md.

stated, that plasmodia and Vorticellidæ were watched for periods which varied from one to fifteen days; large plasmodia were isolated or preserved in concave slides. Even on plane slides the pressure of the cover slip was slight enough to allow of the emission of short pseudopodia in planes at right angles to the plane of extension of the slide and the animals, by means of pipettes, were transferred to fresh water daily.

In a synopsis of the work of Greenwood and Saunders, in a previous report bearing on this matter, in the Journ. of Physiol., the changes undergone by litmus, Congo red and alizarin sulphate, and the solution of the globoids of aleurone grains, which are composed of a delicate nitrogenous capsule enclosing pure calcium and magnesium phosphate, were described. It was emphasized that the outpouring of acid is unaccompanied by any digestive change on nutritive matter; ingesta may indeed be stored for many hours in vacuoles before they are dissolved, or digestion may follow rapidly on ingestion. But the formation of the digestive vacuole, whether immediate or delayed, is preceded by the development of acid reaction and followed by its diminution. Bearing in mind that litmus is changed from blue to red not only by free acid, but also by unsaturated compounds of acid with the products of digestion, *i. e.*, acid salts. And that Congo red changes to blue in presence of free acid only. It is apparent that the diminution of acid in a digestive vacuole is at first due to a combination with the products of digestion, for at this stage any litmus accompanying ingesta is still red, while Congo red has reverted to that tint from blue. Here free acid is absent but acid salts are present. But later on the vacuoles and ingesta, reddened by litmus, become violet and blue so that finally acid and acid combinations are alike absent. That the acid is at one time free is indicated unmistakably by the striking development of violet colors in solids stained with Congo red. Now as the amount of acid present at any moment must be very small, and this being so, that the change in Congo red should be speedy and striking suggests that it is an inorganic acid but it is probable that to emphasize such an inference would be hasty.

In most of the existing records of Protozoan digestion there are indications that the process shows irregularity in its outset and progress. It is not easy to foretell the immediate fate of ingested matter though of its ultimate fate there may be little doubt. There may be marked inhibition of digestive activity even after free ingestion. In plasmodia ingested nutrient matter may be actually discharged after very imperfect digestion. One of the most puzzling phenomena, however, that has been described by all observers in this field, has been termed by Greenwood and Saunders the stage of storage. This process consists in the preservation of ingested food masses, which on first enclosure have been surrounded by liquid within a vacuole, in a shrunken seemingly very acid state. At times 100 non vacuolate, acid ingesta may lie within the substance of a vorticella, whilst active digestive solution is going on in other food vacuoles at same time.

The storage of nutritious ingesta for hours and days in a condition in which acid indicators give evidence of an acid condition, whilst the same kind of nutritious material will undergo rapid digestive solution in an adjacent vacuole, naturally excites one's curiosity. For a long time I had been looking in vain for some explanation of this phenomenon when an accident gave opportunity of viewing it in a new light.

The plasmodia of a large mycetozoon, most probably *Lamproderma scintillans*, had been under observation for about three weeks. Some of these amoeboid organisms were so large as to more than cover the field of vision when objective D and apochromatic eye-piece, No. 4 of Zeiss were used. They showed a habit of devouring everything in their vicinity in the ditch water in which they were cultivated, as a result of which they were at times so filled with debris that no accurate observations were possible. It was planned to transfer them gradually by pipettes into clearer and clearer water and by starvation compel them to rid themselves of the dirt they contained. This proved successful and after 8–18 days of transferring the plasmodia were in practically clear water, free from algæ, infusoriæ, gregarines, bacteria, etc., and the usual

fauna and flora of ditch water. It was a surprise to find that dried egg albumen stained or ingested with litmus and Congo red, under these new conditions, was as a rule promptly dissolved in the vacuoles, taking from 5–24 hours for completion of the digestive act. The same occurred with stained globoids of aleurone grains of ricinus and with stained torulæ. These experiments were repeated many times on many different individuals, and though food ingesta were occasionally observed in a stage of storage, this was the great exception.

The scarcity of storage vacuoles in such plasmodia that had been kept in clear water for nearly a week and given opportunity to disgorge the debris with which they were loaded was conjectured might be brought about by two factors:

(1) The first was that the process of clearing and transferring them to distilled water (in which they do not thrive as well as in Pasteur's fluid with $\frac{1}{5}$ % Na cl) the organisms had been starved and in a sense were too hungry to store food particles, but went to work at them immediately. There is no method conceivable by which such a supposition could be put to experimental test, for which reason it cannot be contradicted or proved.

The second supposition was that (2) absence of storage vacuoles might be caused by absence of bacteria, for in their normal environment the Protozoa are generally in close company with swarms of *Bacierium termo*, zooglea of micrococci and manifold spirilli and other schizomycetes, and by cultivation they had been brought into an almost aseptic, sterile environment.

The latter hypothesis is capable of experimental testing. For if bacteria will produce the phenomenon of storage then the supplying of septic food will be all that is requisite to add to the sterile solution. As a matter of fact it will be found that this is exactly what will happen. In a plasmodium that had shown 8 storage vacuoles in 24 hours of observation in a solution of $\frac{1}{2}$ % sodium chlorde (in distilled water) in which it had been kept one week, 48 storage vacuoles were observed in the next 10 hours on supplying dried albumen dust, moistened with the zoogloea from a Hay infusion.

Vorticellidæ which take in food particles readily are remarkably free from bacteria in their food vacuoles. Amoeba and plasmodia alike exercise to some extent a selective ingestion. Greenwood and Saunders claim to have watched *Amoeba proteus* for 14 days when surrounded with *Bact. termo*, vibrios and micrococci and the absence of bacteria from the endosarc was remarkable. They are taken in, it would seem, as unavoidable accompaniments of surrounding food only. Bacteria are not recorded to have been observed ingested by protozoa per se.

Another evidence of selective ingestion has been mentioned by Dantec, *l. c.*, as distinguishing between inert and living matter. Active monads or groups of spirilli are placed in marked vacuoles of ingestion, containing much of the acid secretion in comparison to inert matter which is usually invested very closely. We therefore have some evidence for assuming that plasmodia and Vorticellidæ distinguish between inert food and bacteria.

(1) Bacteria are rarely ingested except as unavoidable accompaniment of food. (2) Inert food, free from bacteria, is invested closely. Septic food within wide vacuoles. (3) In sterile environment, food in the stage of storage is the exception; in environment of bacteria, storage in acid vacuoles is frequent. I have brought these facts before you in this incomplete form, because the results are fairly uniform, and with the hope of stimulating further observation of the matter. These studies require no apparatus outside of the microscope and acid indicators. The general suggestion drawn from the result has a wider bearing than one would at first sight assume. For if further study will confirm that the ingestion of bacteria constantly prolongs the stage of maximum acidity from the usual time of 24 hours to several days in rhizopods. The suggestion is that the purpose of the acid is one of (disinfection) killing off bacteria.

There is a general uniformity of opinion that the presence of acid is unaccompanied by any digestive change on nutritive matter, which may be stored for many hours before it is dissolved and Greenwood and Saunders intimate that the endo-

sarc secretes some zymogen which perfects the digestive secretion.

The object to which the acid would seemingly serve in these organisms, which may be said to be on the very threshhold of life is the same which Bunge ascribes to it in man. Bunge's view is that the HCl has no other purpose than the sterilization of food. "Why should a chemical substance be placed in the entrance to the digestive tract," he asks, "in exactly the strength necessary for the destruction of bacteria which is directly antagonistic to the chemical reaction in which the main work of digestion must be carried on? The proteids are more readily converted into a solution lower down in the intestine and in an alkaline medium than by pepsin and acid. The object of the acid is, according to him, then, one of sterilization. This view cannot be denied, at the same time it must be admitted that HCl serves also a digestive purpose.

In the Rhizopods experimented upon, the observations of Greenwood and Saunders could be confirmed concerning the fact that while the acid is secreted in the food vacuoles under the stimulus of all ingesta; the true digestive vacuole which occurs only under the stimulus of nutritive matter apparently contains something besides an acid, perhaps an enzyme. The change in the acid indicators is as regards time and intensity of color transformation to all observation alike. There seems to be the same amount of acid in a storage vacuole as in a vacuole causing active solution of proteid matter, in close proximity to it, hence the assumption of an additional zymogenic substance in the latter is justifiable. As the amount of acid in one of these vacuoles is very small, and the change in Congo red to blue is speedy and striking, lends belief to the suggestion of Greenwood that the acid is an inorganic one. Why the protoplasm around a storage vacuole will not secrete zymogenic matter, though acid is clearly present in it, and at the same time this enzyme must be accepted to be present in a vacuole in which, close to the former, active digestion is going on is a question difficult to approach. If it can be demonstrated that all or most storage vacuoles contain some substance, living or inert, which is hostile to the economy of the Rhizopod and against which it protects itself by intensely acid

investment of the enemy for a prolonged period, a new and interesting light will be thrown on this phenomenon.

In the "Centralblatt für Bacteriologie, Parasitenkunde u. Infektions krankheiten, Vol. XIX, p. 785, Dr. C. Gorini describes a method for cultivating *Amoeba zymophila* on a solid medium which in this case is the potato. It is certain that Amoebae will grow on old and new potatoes with alkalinization. This would offer an easy and convenient method of cultivating them. It should be emphasized that it is almost impossible to produce cultures of amoeba that are absolutely free from bacteria. A. Celli in the Centralbl. f. Bacteriologie, Bd. XIX, p. 537, describes a number of futile attempts to obtain such cultures. For our purpose it is not essential that the amoebic cultures should be absolutely free from bacteria, a relative, approximate sterility is sufficient to demonstrate the scarcity of storage vacuoles in the amoebae and plasmodia in such environment. Celli's favorite solid medium is a preparation made from Fucus Crispus with 5 per cent Sterilized Water, with or without Bouillon, but always made alkaline. To 10 c.c. culture medium, 1 c. c. of an $\frac{N}{10}$ Solution of Potassium hydroxide or 4–5 c. c. of a saturated solution of Sodium Bicarbonate. This culture medium of Fucus after it is made in the manner that Agar is generally prepared solidifies readily.

In the same Journal, Centrbl. für Bacteriologie, Band XIX, p. 258, Dr. M. W. Beyerinck describes a solid medium for amoebic cultures made from solidified agar by diffusion of the soluble organic substances in it into superimposed distilled water, which process requires about two weeks and repeated sterilization and subsequent addition of salts suitable to formation of nitrites.

I have no experience with these methods and have always found that for my purpose a solution of a little wheat bread in distilled water kept in a small flat dish under a glass cover was all that was required to have Amoeba and plasmodia of mycetozoa constantly on hand. The dish must be kept on a little earth and not in too bright a light and at a constant temperature. This simple culture medium, which of course is unsuitable for pure cultures was suggested by Prof. Reichert of the University of Pennsylvania.